Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytica Chimica Acta 509 (2004) 111–118
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Monoclonal antibody-based enzyme-linked immunosorbent assay for the insecticide imidacloprid

Hee-Joo Kim [a], Weilin L. Shelver [b], Qing X. Li [a,*]

[a] *Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI 96822, USA*
[b] *Biosciences Research Laboratory, Agricultural Research Service, United States Department of Agriculture, 1605 Albrecht Boulevard, Fargo, ND 58105-5674, USA*

Received 28 May 2003; received in revised form 19 November 2003; accepted 4 December 2003

Abstract

An enzyme-linked immunosorbent assay (ELISA) was developed for the neonicotinoid insecticide imidacloprid, 1-[(6-chloro-3-pyridinyl)-methyl]-*N*-nitro-2-imidazolidinimine using monoclonal antibodies (MAb). Three MAbs, designated as E6A6, E6F3 and H7F7, were raised from mice immunized with an imidacloprid hapten–ovalbumin conjugate. These MAbs performed similarly in indirect competition ELISA (icELISA), so one, E6F3, was selected for detailed study. The equilibrium constants (K_d) and association and dissociation rate constants (k_{on}, k_{off}) for five neonicotinoids and one imidacloprid metabolite to E6F3 were determined by kinetic exclusion fluoroimmunoassay (KinExA). Affinities ($1/K_d$) of E6F3 for acetamiprid and clothianidin were similar, but 50-fold weaker than that of imidacloprid. MAb E6F3 had no measurable affinity for the other neonicotinoids. The icELISA can tolerate up to 15% (v/v) acetone or 20% (v/v) methanol. Assay sensitivity was similar at pH 4–9, 1–10-fold concentration of PBS with or without 0.05% Tween 20, and incubation times of 30–180 min. The half-maximal inhibition and the limit of detection were approximately 0.8 and 0.1 μg/l of imidacloprid in icELISA, and 0.3 and 0.03 μg/l in direct competition ELISA (dcELISA), respectively. Analysis of imidacloprid-fortified water and cucumber samples by the icELISA showed average recoveries from 70 to 120%.

Keywords: ELISA; KinExA; Immunoassay; Neonicotinoid insecticide; Imidacloprid

1. Introduction

Imidacloprid, 1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine, belongs to a relatively new class of insecticides known as neonicotinoids [1–4]. Imidacloprid and other neonicotinoids act as agonists on the postsynaptic nicotinic acetylcholine receptors (nAChR), causing the modification of insect behavior and finally death [5,6]. Research on the molecular basis for the selectivity of neonicotinoids has elucidated the mechanism of nAChR–insecticide interaction and suggested that the low mammalian toxicity can be attributed to the higher selectivity of neonicotinoids for insect nAChR than for vertebrate nAChR [4,7–16]. Because neonicotinoids have greater systemic activity, lower acute mammalian toxicity, and no cumulative long-term toxicity, they may eventually replace pyrethroid, organophosphate and carbamate insecticides. However, several properties of neonicotinoids, such as wind drift, leaching into surface water and ground water, and toxicity to honey bees and other beneficial organisms, remain to be elucidated [17–22].

Two chemical classes of neonicotinoid insecticides are currently in use. These are the chloropyridinyl derivatives such as imidacloprid, and the chlorothiazolyl derivatives, exemplified by thiamethoxam. High performance liquid chromatography (HPLC) with UV detection or HPLC–mass spectrometry (HPLC–MS) are currently preferred for the determination of imidacloprid in environmental samples [23–29]. The thermolability and high polarity of neonicotinoids make them difficult to analyze by gas chromatography (GC) or GC–MS, which requires derivatization of the analytes prior to analysis [30–32].

Immunoassay, however, has proven to be a good analytical method for rapid monitoring of agrochemicals [33,34]. To date, several enzyme-linked immunosorbent assays

* Corresponding author. Tel.: +1-808-956-2011; fax: +1-808-956-3542.
E-mail address: qingl@hawaii.edu (Q.X. Li).

0003-2670/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2003.12.006

(ELISAs) have been developed for neonicotinoids. We have reported two ELISAs for imidacloprid and thiamethoxam that use rabbit antisera [35–37]. Lee et al. [38] developed another ELISA for imidacloprid based on rabbit sera, and Wanatabe et al. [39] derived two monoclonal mouse antibodies for an ELISA of imidacloprid and acetamiprid. In this paper, we describe the development of an ELISA for imidacloprid based on a new mouse MAb derived from Hapten II that we described previously [35]. We also present solution-phase kinetics of the MAb binding to imidacloprid, and other neonicotinoids, determined using kinetic exclusion assay (KinExA).

2. Experimental

2.1. Materials and reagents

All reagents were of analytical grade unless specified otherwise. Reference standards of clothianidin (99.9%), acetamiprid (99.5%), and dinotefuran (99.7%) were kindly provided by the National Institute of Agricultural Science and Technology, South Korea. Imidacloprid and the imidacloprid haptens were synthesized in this laboratory as previously described [35]. Chemicals purchased from Sigma (St. Louis, MO, USA) were goat anti-mouse IgG-horseradish peroxidase (IgG-HRP), bovine serum albumin (BSA), ovalbumin (OVA), keyhole limpet hemocyanin (KLH), phosphate–citrate buffer capsules with sodium perborate, carbonate–bicarbonate buffer capsules, *o*-phenylenediamine (OPD), complete and incomplete Freund's adjuvant, DMSO, polyethylene glycol 1500 (PEG), and goat anti-mouse IgG, *N*-hydroxysuccinimide (NHS), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC). 3,3′,5,5′-Tetramethylbenzidine (TMB) was obtained from Kirkegaard & Perry Labs. (Gaithersburg, MD, USA). The monoclonal antibody isotyping kit was purchased from Pierce (Rockford, IL, USA). HT, HAT and RPMI 1640 supplements were bought from Life Technologies (Grand Island, NY, USA). Fetal bovine serum from Hyclone (Logan, UT, USA) was heat inactivated at 56 °C for 30 min prior to use. Hybridoma cloning factor (ORIGEN) was obtained from Fisher Scientific (Pittsburgh, PA, USA). Murine myeloma cell line Sp2/0Ag14 was purchased from the American Type Culture Collection (Manassas, VA, USA; ATCC Catalog No. CRL-1581). The ELISAs were carried out in 96-well polystyrene microplates (MaxiSorp F96; Nalge Nunc International, Copenhagen, Denmark). The MAb was purified with a T-gel purification kit (Pierce) according to the manufacturer's instructions. Concentrations of purified antibody were determined with the Bio-Rad Bradford protein assay (Bio-Rad Labs., Hercules, CA, USA). The purified IgG in phosphate-buffered saline (PBS, 5 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 136 mM NaCl, and 2.7 mM KCl, pH 7.5) was stored at −20 °C until use.

2.2. Preparation of protein–hapten conjugates

Imidacloprid haptens (Fig. 1) were conjugated to KLH, OVA, and BSA according to the procedures previously described [35], but scaled down by 50% (hapten 0.05 mmol, NHS 0.1 mmol and EDC 0.1 mmol) in 0.5 ml DMF. All the conjugates were stored at −80 °C. Aliquots of conjugates were stored at 4 °C for daily use.

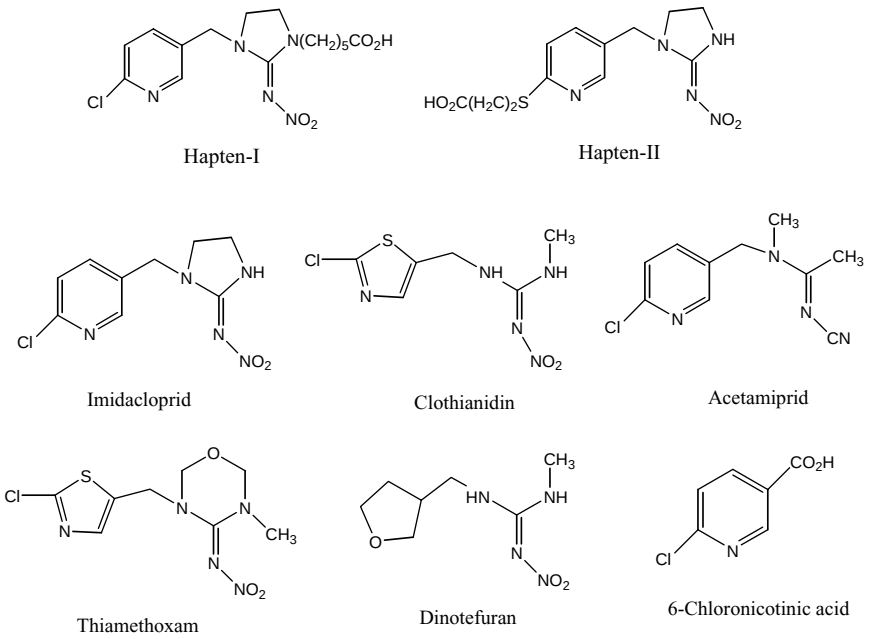

Fig. 1. Structures of imidacloprid haptens, imidacloprid, its major metabolite 6-chloronicotinic acid and other neonicotinoids.

2.3. Monoclonal antibody generation and characterization

The procedures for generating the immune response in mice and producing MAbs were similar to those described by Shelver et al. [40]. Five female Balb/c mice, 6 weeks old (Jackson Labs., Bar Harbor, ME, USA), were initially immunized by intraperitoneal (i.p.) injection with 50 μg of imidacloprid hapten I–KLH or hapten II–OVA. After 4 monthly i.p. booster immunizations with 50 μg of hapten conjugate emulsified with incomplete Freund's adjuvant, serum samples were taken. One mouse, immunized with hapten II–OVA, developed a serum antibody titer >1:30,000 against hapten II–BSA, and the strongest competitive binding of soluble imidacloprid in an icELISA. This mouse was selected for hybridoma production. Four days prior to splenocyte harvest, this mouse was injected with the hapten II–OVA in PBS, 50 μg through the tail vein and 50 μg i.p.

Murine Sp2/0Ag14 myeloma cells were grown in RPMI 1640 supplemented with 15% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (designated as complete medium). The culture medium supernatants were saved from each Sp2/0Ag14 cell splitting and used as the conditioned medium replacing feeder cells in the fusion and cloning experiments. Splenocytes were harvested from the best-responding mouse described above, hybridomas were prepared by fusion with the Sp2/0Ag14 myelomas, and selected in complete medium containing 10 μM sodium hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine (HAT) as previously described [40]. After plating in 96-well culture plates, the HAT selection was performed from 2 to 14 days postfusion. Two weeks after fusion the cells were screened for their ability to produce antibodies to imidacloprid utilizing concurrent indirect non-competitive and competitive ELISAs with TMB as a substrate. One hybridoma colony designated 6C9 was selected for further study, based on its competitive binding and ability to withstand expansion and preservation in liquid nitrogen. The 6C9 cells were cloned twice by limiting dilution in complete medium with 10% ORIGEN cloning supplement. Four clones, designated E6A6, E6F3, H7F7, and H7A7, were expanded and archived for further study. Isotyping, performed with a commercial kit (Pierce, Rockford IL, USA), showed that all four clones were $IgG_1\kappa$. Unless otherwise specified, all experiments were done with MAb E6F3 culture supernatant.

2.4. Assay optimization

Effects of assay buffer ionic strength, pH, Tween 20, and solvents were done according to the procedures previously described [36]. In addition, plates were incubated after mixing equal volume of MAb and imidacloprid standard diluted in PBST at various time periods (30, 60, 90, or 180 min) to estimate the effect of incubation time. The remainder of the ELISA procedures was done under optimized conditions.

2.5. icELISA

Microplate wells were coated with hapten I–BSA or hapten II–BSA (4 ng in 100 μl per well in 0.05 M carbonate–bicarbonate buffer, pH 9.6) overnight at 4 °C. The following day, the plates was washed four times with PBS containing 0.05% Tween 20 (PBST) and then blocked with 1% BSA in PBS (200 μl per well) by incubation for 1 h at room temperature. The plates were washed again five times, a solution of 50 μl per well of analytes or standard diluted in PBST and 50 μl per well (0.1 μg antibody per well) of imidacloprid MAb was added and incubated at 37 °C for 40 min. Peroxidase-labeled goat anti-mouse IgG (1:10,000 in PBST; 100 μl per well) was then added, and the plates were incubated 40 min at 37 °C. The plates were again washed five times as above, and then substrate solution (100 μl per well of 0.05 M citrate–phosphate buffer, pH 5.0, containing 0.03% sodium perborate, and 1.0 mg/ml of OPD) was added. After 20 min at room temperature, the reaction was stopped with sulfuric acid (4N, 50 μl per well), and absorbance at 490 nm was read with a Vmax microplate reader (Molecular Devices, Sunnyvale, CA, USA). Samples and standards were generally analyzed in four replicate wells. Inhibition curves were fitted with the four-parameter logistic equation using Softmax version 2.35 software (Molecular Devices).

2.6. dcELISA

The dcELISA was done as previously described [37]. Hapten II–HRP conjugate was used as an enzyme tracer.

2.7. Measurements of binding kinetics

Binding kinetics were determined using kinetic exclusion fluoroimmunoassay (KinExA). The principles, and details of the KinExA 3000 instrument (Sapidyne Instruments, Boise, ID, USA) and assay procedures have been described elsewhere [41–45]. In the experiments reported here, polystyrene beads of 98 μm diameter were obtained from Sapidyne Instrument Inc. Aliquots of dry beads (200 mg) in 1.5 ml Eppendorf tubes were suspended and settled twice in 1 ml of 6N NaOH, and then washed successively in glass distilled water, PBS, and coating buffer. The beads were suspended in 1 ml of coating buffer containing 0.2 mg of hapten II–BSA and the tube gently rolled at room temperature for 3 h. After the conjugating solution was discarded, the beads were washed three times with PBS, resuspended in blocking buffer (1% BSA in PBS), and mixed by rolling at room temperature for 2 h. Beads were washed with PBS three times again and stored at 4 °C until they were used for assay. On the day of use, 200 mg aliquots of coated beads were uniformly suspended in 27 ml of PBS. For each sample analyzed, a new bed of hapten conjugate-coated beads exactly 4 mm high (to match the width of the excitation beam) was deposited over the mesh trap in the capillary flow cell. To determine K_d, various concentrations of analyte and a

fixed amount of the MAb solution were mixed and allowed to equilibrate for 1 h at room temperature. The solutions were passed through the KinExA's beads to capture MAb with unoccupied binding sites on the immobilized hapten. After a brief wash, (250 μl in 30 s) a solution of Cy5-labeled goat anti-mouse IgG was passed through the beads, and after a brief wash, the fluorescence of the bound secondary antibody conjugate was used to quantify the primary antibody. To measure k_{on} a constant amount of antibody and various concentrations of an analyte were mixed by simultaneous injection, and the mixture was passed through an injection port for 17.5 s, and then through the capillary containing the haptenated beads, at a predetermined rate. After washing with PBS, the amount of bound primary antibody on the beads was quantified with Cy5-labeled goat anti-mouse IgG, as in the K_d determination. The off rate $k_{off} = k_{on}K_d$. Detailed calculations for determination of the amount of functional MAb, and all other parameters, including standard errors, may be found in [45].

2.8. Fortification of imidacloprid in water and cucumber

Water samples were collected from a tap in the laboratory and from the Manoa stream in Honolulu, HI. Aliquots were fortified with imidacloprid to 0, 2, 10, 50, and 200 ng/ml, mixed with an equal volume of two-fold concentrated PBST (24 mM phosphate, 274 mM NaCl, 5.4 mM KCl, 0.1% Tween 20, pH 7.5) containing a pre-determined limiting amount of MAb. Cucumbers were purchased from a local grocery store and homogenized to a free-flowing puree in a blender (Waring, USA). Aliquots (10 g) of the homogenate were fortified with imidacloprid standard in DMSO to 0, 10, 20, 50, 200, and 500 ng/ml. The fortified cucumber samples were extracted with 20 ml of MeOH for 30 min, and then centrifuged for 20 min at 10,000 rpm to remove the solids. The supernatants were filtered through a 0.45 μm membrane. The sample extracts were analyzed with icELISA.

3. Results and discussion

3.1. Characterization of MAbs

Groups of mice were immunized with one of two different haptens (Fig. 1) with the linkers attached to different ends of the molecule. Hapten I has a linker on the nitro imidazolidinyl ring exposing the 6-chloropyridinyl moiety. In hapten II, the linker is on the pyridinyl ring, which presents the nitro imidazolidinyl moiety for antibody recognition. Two ELISAs for imidacloprid and two major metabolites (imidacloprid olefin and 5-OH-imidacloprid) were previously developed with antisera raised with these two haptens [35]. This study was expected to produce new MAbs for imidacloprid and possibly some of its metabolites. However, none of the mice immunized with hapten I–KLH conjugate produced MAb able to compete with imidacloprid. Four MAbs derived from the mouse immunized with hapten II–OVA were tested for their competitive binding to imidacloprid in icELISA with each of the haptens. As shown in Table 1, none of the MAbs showed competitive inhibition with hapten I–BSA. Although all four MAbs were originally selected for binding to hapten II–BSA, three competitively recognized imidacloprid, and their I_{50} values were identical within experimental error. Thus, the four clones represented a minimum of two cell lines. MAb H7A7 cannot be used for competition ELISAs, but may prove useful for applications such as immunoaffinity cleanup of imidacloprid. Hybridoma lines E6A6, E6F3, and H7F7 produced MAbs with the same IgG1κ isotype and I_{50} values. These cell lines may be identical clones, although this was not tested in other ways. Only supernatant from hybridoma E6F3 was used for the remainder of this study.

Table 1
Competitive binding of MAbs to imidacloprid haptens

Coating antigen[a]	I_{50} (μg/l)			
	E6A6	E6F3	H7F7	H7A7
Hapten I–BSA	nc[b]	nc	nc	nc
Hapten II–BSA	14.3 ± 0.4[c]	14.6 ± 0.8	15.2 ± 0.6	nc

[a] The coating antigen was at 5.0 ng in 100 μl per well.
[b] No competitions were observed up to 5000 ng/ml of imidacloprid.
[c] I_{50} values obtained in icELISA with hybridoma culture fluid diluted 1:100 in PBST. Each value represents the mean of four replicates ± S.E.

3.2. Competitive inhibition

Fig. 2 shows representative standard curves for imidacloprid generated by the icELISA and dcELISA. The working ranges of the icELISA and dcELISA were approximately 0.1–4.0 and 0.03–4.0 μg/l, respectively. The I_{50} values of icELISA and dcELISA were 0.8 and 0.3 μg/l, respectively. Although use of dcELISA format gave a nearly 3-fold lower

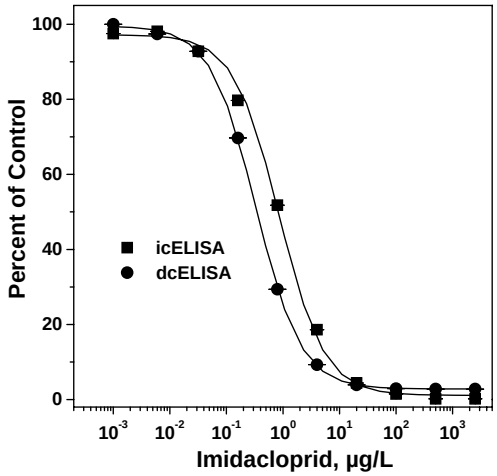

Fig. 2. Standard inhibition curves of imidacloprid in dcELISA and icELISA formats. Plates were coated with 0.5 μg of purified MAb for dcELISA or 4.0 ng of coating antigen per well for icELISA. Each value represents the mean of four replicates.

I_{50} value than that of icELISA, this improvement is small compared with our previous observations where dcELISA improved assay sensitivity 20-fold [36,37]. Wanatabe et al. [39] produced three different MAbs using the same hapten as the one used in this experiment. Their ELISAs showed I_{50} values from 0.8 to 6.4 μg/l and low cross reactivity to two neonicotinoids and some metabolites. Lee et al. [38] synthesized two different haptens to raise imidacloprid antisera. One had a linker attached via the nitro group and the other had the same structure as hapten I used in this experiment, except for differences in the length and functional group of the linker. The former hapten failed to produce imidacloprid specific antisera in rabbits, indicating that the nitro guanidine (=N–NO_2) moiety may act as a key antigenic determinant. The latter hapten elicited antisera that competitively bound imidacloprid with an I_{50} of 17.3 μg/l. The I_{50} of our dcELISA was approximately 3- and 20-fold lower than that of Wanatabe et al.'s and Lee et al.'s, respectively.

3.3. Physicochemical effects on assay performance

Immunoassay performance is often affected by chemical parameters such as ionic strength, pH, surfactant, organic solvent concentration, and substances in the sample matrix, as well as physical factors such as incubation time and temperature at different assay steps. The effects of these parameters were estimated by comparing I_{50} values obtained under various conditions with that of a control. The maximum absorbance (A_{max}), reflecting maximal binding to the competing hapten and the lowest I_{50} were observed at pH 7 (Fig. 3). Although A_{max} values were lower at pH less than 6 and greater than 8, there were no significant changes in I_{50} values for assays run between pH 5 and 8. All subsequent assays were performed at pH 7.0. No significant changes in I_{50} values and A_{max} were observed over 1–10-fold concentrated PBS containing 0.05% Tween 20 (data not shown). In practice, ionic strength of environmental samples can be adjusted by simple dilution with water or concentrated

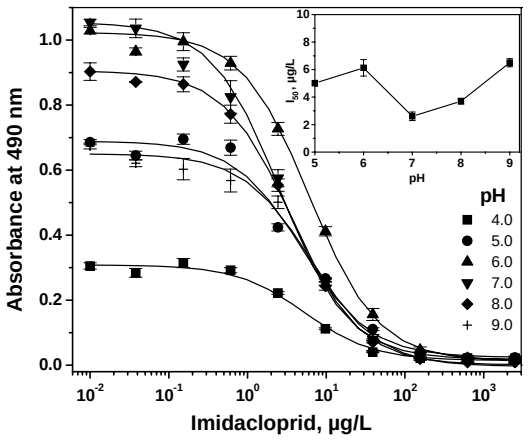

Fig. 3. Effects of assay buffer pH. Each solid symbol represents the mean of four replicates.

buffer. Although PBS with or without 0.05% Tween 20 showed no effect on assay sensitivity and A_{max}, addition of Tween 20 slightly reduced variations in absorbance among replicate wells so that Tween 20 at 0.05% was present in all assays. Incubation times of 30–180 min for the competition step did not change assay performance (data not shown) so incubation at 37 °C for 40 min was adopted. In addition, the effects of DMSO, MeOH, acetone and acetonitrile were studied because these solvents are water-miscible and are commonly used in sample extractions. Fig. 4 shows normalized dose–response curves at various solvent concentrations. In general, little effects of four solvents on A_{max} were observed when the assay was run in concentrations of solvents up to 20%. However, I_{50} values increased gradually as concentrations of acetonitrile and DMSO increased. MeOH and acetone showed less effect on assay sensitivity (I_{50}) than acetonitrile and DMSO. I_{50} was negligibly affected by MeOH up to 20%, but it was approximately doubled in 20% acetonitrile. The presence of 20% DMSO resulted in a 38% drop in the A_{max}. The I_{50}, however, did not change appreciably. Although we observed some effect of acetonitrile and DMSO on assay sensitivity, we still obtained reproducible inhibition curves and usable I_{50} values. Thus, this assay accurately determines the concentration of imidacloprid in solvent extracts with less need for dilution. By comparison, the imidacloprid polyclonal antibody (PAb) was significantly affected by acetone and acetonitrile, but much less by MeOH and DMSO [36].

3.4. Kinetics of MAb binding and cross reactivity

Table 2 shows the K_d, k_{on}, and k_{off} values determined by KinExA for the MAb E6F3. Also presented for comparison are I_{50} data obtained with icELISA. MAb E6F3 was very specific for imidacloprid with a cross reactivity of <4.0% with other neonicotinoid insecticides (Table 2). The K_d values determined by KinExA for clothianidin and acetamiprid were very similar, despite a large difference in I_{50} values for these compounds in icELISA. Dinotefuran, thiamethoxam and 6-chloronicotinic acid were not detectable by MAb E6F3 in KinExA or ELISA. The on-rate (k_{on}) of imidacloprid, which was directly measured by KinExA, was approximately 10 times faster than that of clothianidin or acetamiprid. The k_{off} value of imidacloprid (calculated from K_d and k_{on}) was approximately 5 times slower than that of clothianidin and acetamiprid. This was somewhat unusual, because antibodies generally bind small molecules with similar, fast on-rates and different off-rates [46].

The KinExA and ELISA results may be explained in part by the computational models of the neonicotinoids and haptens that we described previously [37]. The weak affinity of MAb E6F3 for clothianidin and acetamiprid is likely due to lack of the imidazolidine ring, which probably accounts for much of imidacloprid's binding. The 2-chlorothiazole ring of clothianidin is electronically and sterically very similar to the chloropyridine ring of imidacloprid or acetamiprid and

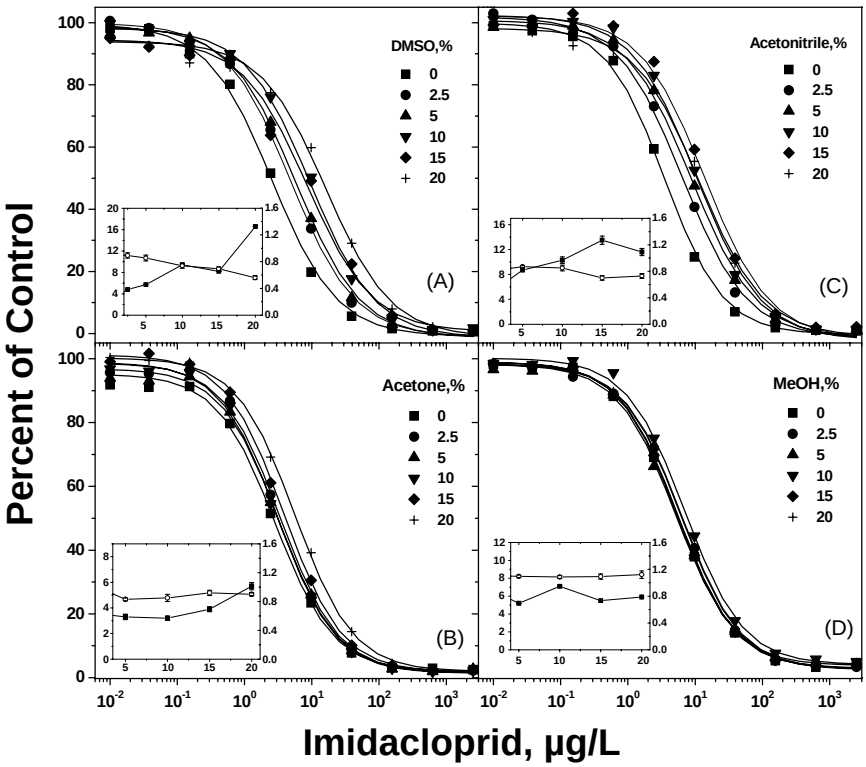

Fig. 4. Effects of DMSO (A), acetone (B), acetonitrile (C), and MeOH (D) on the assay. Values refer to the final concentrations of solvents (v/v) in the competitive assay solution. Insets indicate the fluctuations of A_{max} (right *Y*-axis) and I_{50} (left *Y*-axis) ((○) A_{max}; (■) I_{50}) as a function of solvent concentration (*X*-axis). Each value represents the mean of four replicates.

could be reasonably expected to occupy the same binding site as imidacloprid. The computational modeling suggested that steric constraints of the thiadiazinane ring prevent the nitro group of thiamethoxam from lying in the plane of the thiadiazinane ring as it is able to in imidacloprid and acetamiprid. The nitro group of thiomethoxam would be oriented perpendicularly to the thiadiazinane ring, so that the dihedral angle of the nitro group in thiamethoxam would be quite different from that in imidacloprid [37]. Clothianidin was bound weakly by MAb E6F3, possibly due to the similar alignment of its nitro group in its structure as that of thiamethoxam. Dinotefuran has a tetrahydrofuran group that does not resemble the chloropyridine groups in imidacloprid electronically or sterically. This may explain why MAb E6F3 does not bind dinotefuran.

3.5. Imidacloprid-fortified water and cucumber samples

Tap and natural stream water and cucumber samples spiked with imidacloprid were analyzed by icELISA. Ionic strength of the samples was adjusted by addition of an equal volume of two-fold concentrated PBST containing a fixed amount of MAb. The recovered concentrations of imidacloprid by ELISA correlated well with the spike concentration, with a correlation coefficient of 0.99 for both water and cucumber samples and with overall mean recovery ranged

Table 2
Binding characteristics of MAb E6F3 to neonicotinoid insecticides and 6-chloronicotinic acid

Compound	KinExA			ELISA		
	K_d (nM)	CR[a] (%)	k_{on} ($M^{-1}\,s^{-1}$) × 10^6	k_{off} (s^{-1})	I_{50} (nM)	CR (%)
Imidacloprid	0.7 ± 0.17	100	14.40 ± 3.96	0.010	6.2	100
Clothianidin	35.7 ± 3.4	2.0	1.63 ± 0.085	0.058	175.1	3.6
Acetamiprid	44.6 ± 9.5	1.6	1.16 ± 0.113	0.052	1078	0.6
Thiamethoxam	nc[b]		nc		nc	
Dinotefuran	nc		nc		nc	
6-Chloronicotinic acid	nc		nc		nc	

[a] Cross reactivity.
[b] No competition up to 5.0 μg/ml of each compound.

Table 3
Percentage recovery of imidacloprid fortified to water and cucumber samples by the icELISA

Sample	Imidacloprid (ng/ml)		S.D.[a]	Mean recovery (%, $n = 4$)
	Fortified	Detected		
Tap water	0	nd[b]		
	2	1.8	0.3	92.0
	10	8.6	0.7	85.5
	50	49.4	4.5	98.8
	200	208.0	16.8	104
Stream water	0	nd		nd
	2	2.5	0.4	113.2
	10	10.1	0.9	100.7
	50	52.7	4.5	105.5
	200	239.9	42.6	119.9
Cucumber	0	<0.04		
	10	7.03	0.1	70
	50	39.5	0.2	79
	200	193.8	22.8	97
	500	535.7	19.1	107

[a] Standard deviation.
[b] Not detected.

from 70 to 120% (Table 3). The results showed that the ELISA can accurately measure the concentration of imidacloprid in environmental and food matrices.

4. Conclusion

A sensitive imidacloprid-specific ELISA based on a monoclonal antibody was developed. Separate groups of mice were immunized with the different hapten conjugates, in an attempt to produce MAbs with different specificities. Hapten II–OVA conjugate elicited imidacloprid specific MAbs. However, hapten I–KLH conjugate was not effective in producing a MAb to recognize imidacloprid or its metabolites. KinExA and ELISA revealed different aspects of binding by MAb E6F3. Acetamiprid and clothianidin showed weak binding in icELISA and KinExA. Dinotefuran and thiamethoxam were not bound by E6F3 in either assay, a result consistent with their structural and electrostatic properties. The satisfactory recoveries and correlation between measured and fortified concentrations in two water samples and cucumber homogenate suggest that the assay can be used to quantify imidacloprid residues in these matrices. However, further work will be needed to validate this assay for other applications.

Acknowledgements

The authors would like to thank Grant Harrington for technical support. This work was supported, in part, by the State of Hawaii Department of Agriculture (Pesticides Branch). H.J.K. received a scholarship from the Foundation for Rural Youth, South Korea.

References

[1] S. Kagabu, S. Medej, Biosci. Biotechnol. Biochem. 59 (1995) 980.
[2] S. Kagabu, K. Yokoyama, K. Iwaya, M. Tanaka, Biosci. Biotechnol. Biochem. 62 (1998) 1216.
[3] H.-J. Diehr, B. Gallenkamp, K. Jelich, R. Lantzsch, K. Shiokawa, Pflanzenschutz-Nachrichten Bayer 44 (1991) 107.
[4] K. Matsuda, S.D. Buckingham, D. Kleier, J.J. Rauh, M. Grauso, D.B. Sattelle, Trends Pharmacol. Sci. 22 (2001) 573.
[5] S. Suchail, D. Guez, L.P. Belzunces, Environ. Toxicol. Chem. 20 (2001) 2482.
[6] C. Tharp, G.D. Johnson, J.A. Onsager, J. Econ. Entomol. 93 (2000) 293.
[7] R.J. Lind, M.S. Clough, S.E. Reynolds, F.G.P. Earley, Pestic. Biochem. Physiol. 62 (1998) 3.
[8] K. Matsuda, S.D. Buckingham, J.C. Freeman, M.D. Squire, H.A. Baylis, D.B. Sattelle, Br. J. Pharmacol. 123 (1998) 518.
[9] Y. Huang, M.S. Williamson, A.L. Devonshire, J.D. Windass, S.J. Lansdell, N.S. Millar, Neurosci. Lett. 284 (2000) 116.
[10] M. Tomizawa, J.E. Casida, Toxicol. Appl. Pharmacol. 169 (2000) 114.
[11] S.J. Lansdell, N.S. Millar, Neuropharmacology 39 (2000) 671.
[12] K. Matsuda, M. Shimomura, Y. Kondo, M. Ihara, K. Hashigami, N. Yoshida, V. Raymond, N.P. Mongan, J.C. Freeman, K. Komai, D.B. Sattelle, Br. J. Pharmacol. 130 (2000) 981.
[13] R.J. Lind, D.J. Hardick, I.S. Blagbrough, B.V.L. Potter, A.J. Wolstenholme, A.R.L. Davies, M.S. Clough, F.G.P. Earley, S.E. Reynolds, S. Wonnacott, Insect Biochem. Mol. Biol. 31 (2001) 533.
[14] M. Tomizawa, A. Cowan, J.E. Casida, Toxicol. Appl. Pharmacol. 177 (2001) 77.
[15] M. Shimomura, H. Okuda, K. Matsuda, K. Komai, M. Akamatsu, D.B. Sattelle, Br. J. Pharmacol. 137 (2002) 162.
[16] P. Déglise, B. Grünewald, M. Gauthier, Neurosci. Lett. 321 (2002) 13.
[17] J.H. Thyssen, L. Machemer, in: Proceedings of the 214th ACS National Meeting (AGRO-019), Book of Abstracts, 1997.
[18] R.M. Hollingworth, E.J. Grafius, in: Proceedings of the 225th ACS National Meeting (AGRO-034), Abstracts of Papers, 2003.
[19] E. Gonzalex-Pradas, M.D. Urena-Amate, F. Flores-Cespedes, M. Fernandez-Perez, J. Garratt, R. Wilkins, Soil Sci. Soc. Am. J. 66 (2002) 1821.
[20] Y. Luo, Y. Zang, Y. Zhong, Z. Kong, Chemosphere 39 (1999) 2347.
[21] M. Lambin, C. Armengaud, S. Raymond, M. Gauthier, Arch. Insect Biochem. Physiol. 48 (2001) 129.
[22] C. Cox, J. Pestic. Reform 21 (2001) 15.
[23] A.R. Fernandez-Alba, A. Valverde, A. Agüera, M. Contreras, S. Chirom, J. Chromatogr. A 721 (1996) 97.
[24] S. Baskaran, R.S. Kookana, R. Naidu, J. Chromatogr. A 787 (1997) 271.
[25] M.M. Macke, J. AOAC Int. 81 (1998) 344.
[26] M. Martínez Galera, A. Garrido Frenich, J.L. Martínez Vidal, P. Parrilla Vázquez, J. Chromatogr. A 799 (1998) 149.
[27] A. Garrido Frenich, F.J. Egea González, J.L. Martínez Vidal, P. Parrilla Vázquez, M. Mateu Sánchez, J. Chromatogr. A 869 (2000) 497.
[28] H. Obana, M. Okihashi, K. Akutsu, Y. Kitagawa, S. Hori, J. Agric. Food Chem. 50 (2002) 4464.
[29] C. Blasco, M. Fernández, Y. Picó, G. Font, J. Mañes, Anal. Chim. Acta 461 (2002) 109.
[30] L.M. MacDonald, T.R. Meyer, J. Agric. Food Chem. 46 (1998) 3133.
[31] F.J. Uroz, F.J. Arrebola, F.J. Egea-González, J.L. Martínez-Vidal, Analyst 126 (2001) 1355.
[32] R.G. Lodevico, Q.X. Li, Anal. Lett. 35 (2002) 315.
[33] D.S. Hage, Anal. Chem. 71 (1999) 294R.
[34] J.M. Van Emon, C.L. Gerlach, Environ. Sci. Technol. 29 (1995) 312A.

[35] K. Li, Q.X. Li, J. Agric. Food Chem. 48 (2000) 3378.
[36] J.R. Coats, H. Yamamoto (Eds.), Environmental Fate and Effects of Pesticides, vol. 853, American Chemical Society, Washington, DC, 2003, p. 30.
[37] H.J. Kim, S. Liu, Y.S. Keum, Q.X. Li, J. Agric. Food Chem. 51 (2003) 1823.
[38] J.K. Lee, K.C. Ahn, O.S. Park, S.Y. Kang, B.D. Hammock, J. Agric. Food Chem. 49 (2001) 2159.
[39] S. Wanatabe, S. Ito, Y. Kamata, N. Omoda, T. Yamazaki, H. Munakata, T. Kaneko, Y. Yuasa, Anal. Chim. Acta 427 (2001) 211.
[40] W.L. Shelver, D.J. Smith, E.S. Berry, J. Agric. Food Chem. 48 (2000) 4020.
[41] K.P. O'Connell, J.J. Valdes, N.L. Azer, R.P. Schwartz, J. Wright, M.E. Eldefrawi, J. Immunol. Meth. 225 (1999) 157.
[42] D.A. Blake, A.R. Pavlov, H. Yu, M. Kohsraviani, H.E. Ensley, R.C. Blake II, Anal. Chim. Acta 444 (2001) 3.
[43] Y.W. Chiu, Q.X. Li, A.E. Karu, Anal. Chem. 73 (2001) 5477.
[44] R.M. Carter, R.C. Blake II, T.D. Nguyen, L.A. Bostanian, Biosens. Bioelectron. 18 (2003) 69.
[45] N. Ohmura, Y. Tsukidate, H. Shinozaki, S.J. Lackie, H. Saiki, Anal. Chem. 75 (2003) 104.
[46] D. Weir (Ed.), Handbook of Experimental Immunology, Blackwell, Oxford, 1978, p. 16.1